

Passive Mucosal Immunisation with Novel Simplified IgA Antibodies Produced in Seeds prevents Enterotoxigenic Escherichia Coli (ETEC) Infection in Weaned Piglets

Vikram Virdi¹, Annelies Coddens³, Sam Millet⁴, Sylvie Debuck¹, Bruno Goddeeris³, Eric Cox³, Henri De Greve², Ann Depicker¹. ¹VIB, Ghent, Belgium; ²VIB, Brussels, Belgium; ³Ghent University, Ghent, Belgium; ⁴Institute of Agricultural and Fisheries Research, Melle, Belgium

ETEC related post-weaning diarrhea causes recurrent economic losses to the porcine rearing industry. The embargo on antibiotic prophylaxis has led to a pressing need for a suitable alternative. To evaluate feed based oral prophylactic passive immunization against ETEC, we produced antibodies in seeds of *Arabidopsis thaliana*. Antibodies were designed by grafting 4 variable domains of llama heavy chain 11 antibodies (VHH) against ETEC on the Fc part of porcine IgG and IgA. Transformants producing the 4 VHH-IgG and 4 VHH-IgA antibodies from 0.2% up to 3% of seed weight were obtained. Cotransformation of the VHH-IgA constructs with porcine joining chain and secretory component led to production of assembled dimeric and secretory IgA like antibodies in seeds. *In vitro* analysis of the antibody producing seed extracts were all effective in aggregating ETEC and inhibiting bacterial binding to porcine gut villous enterocytes. In a piglet feed-challenge experiment, the feed containing a milled cocktail of all the VHH-IgA based antibodies (dose 20mg/ pig/ day) protected the piglets against the challenge infection; while feed with the 4 VHH-IgG producing seeds (dose 80mg/ pig/ day) failed to offer similar protection. Piglets receiving the VHH-IgA antibodies had a swift decline in shedding of ETEC, the seroconversion was significantly lower and they had a higher weight gain. Thus these results show a feasibility proof for oral passive immunization against ETEC.